DNA AND PLFA ANALYSES FOR REMEDIATION OPTIMIZATION

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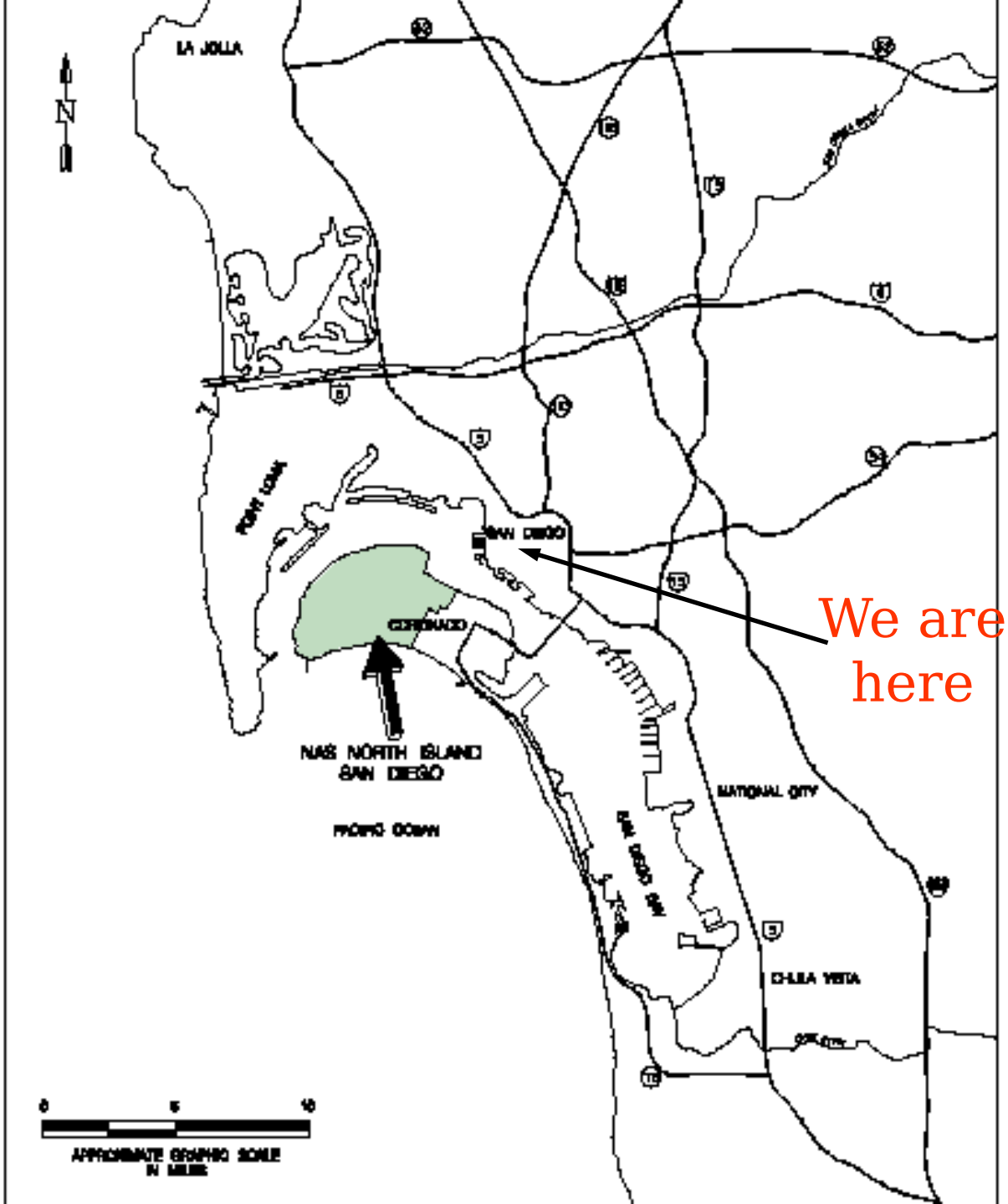

LA JOLLA
N
POINT LOMA
SAN DIEGO
CORONADO
We are here
NAS NORTH ISLAND
SAN DIEGO
PACIFIC OCEAN
NATIONAL CITY
SAN DIEGO BAY
CHULA VISTA
0
5
10
APPROXIMATE GRAPHIC SCALE
IN MILES

Pump & Treat Product Recovery System Facts

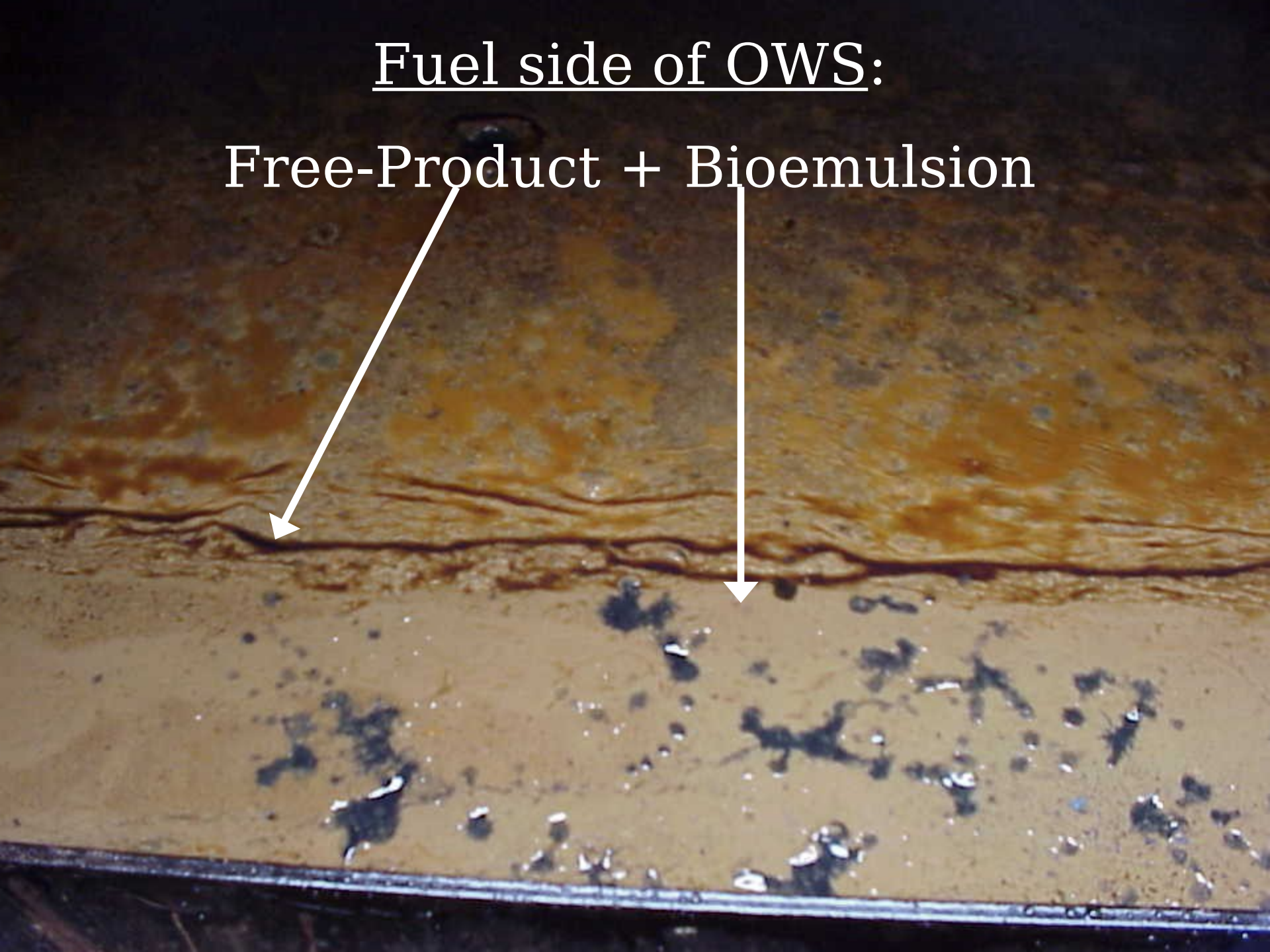
- **Yrs Operational - 7**
- **Plume – 600,000 Gallons Fuel**
- **Fuel Type - JP-5 & AvGasoline**

Project Totals:

- **Fuel Shipped – 239,753 Gallons**
- **Groundwater – 37,840,301 Gallons**

2003 Totals:

- **Fuel Shipped – 20,058 Gallons**
- **Groundwater - 8,769,344 Gallons**


Fuel side of OWS:
Free-Product + Bioemulsion

Biofilm

- Grows on all submersed surfaces throughout remediation system.
- Forms flocs throughout water column.
- Resistant to biocides.
- Problem due to

De-sludging of OWS:

Fuel

Mottled white/gray bioemulsion

Water

Microbial Testing Techniques

Plate Cultures

Phospholipid Fatty Acid Analysis

DNA Analysis

Sample	Heterotrophic Bacterium	Acid Producing Bacterium	Sulfate Reducing Bacterium	Iron Reducing Bacterium	Slime Producing Bacterium
Influent Sample	>500,000 aerobic	25,000	100,000	100,000	500,000
Bioemulsion Sample	>500,000 aerobic	25,000	100,000	100,000	500,000

Reported in Colonies per cc.

The cultures were started on November 22, 2002 and allowed to grow until December 03, 2002.

Why Study Phospholipid Fatty Acids?

- Found in all living organisms.
- Decompose quickly upon cell death.
- Differ between organisms within the microbial community (Phylogenetic identity).
- Nutritional status of the cells (turnover/adaptability).
- Reactions to environmental factors (pollution, etc.).
- Monitoring the microbial responses to their environment.

Slime Sample

Organic Acid Extraction

GC/MS Analysis

Phospholipid Fatty Acid Analysis

- **Viable Microbial Biomass: 7.79×10^7 cells/mL (Very high biomass).**
- **Microbial diversity: Low**
- **Physiological Status: No starvation (low cy/cis ratio) and only moderate membrane stress (moderate trans/cis ratio) – little or no contact with fuel.**

PLFA Community Structure

PLFA Structural Groups:

- **Monoenoic (Monos)-**
- **Normal Saturated –**
- **Term. Branched Saturated –**
- **Branched Monos –**
- **MidBranched Saturated –**
- **Polyenoics –**

Bacterial Class:

67.9% **Proteobacteria (*Zoogloea* & *Sulfuricurvum*).**

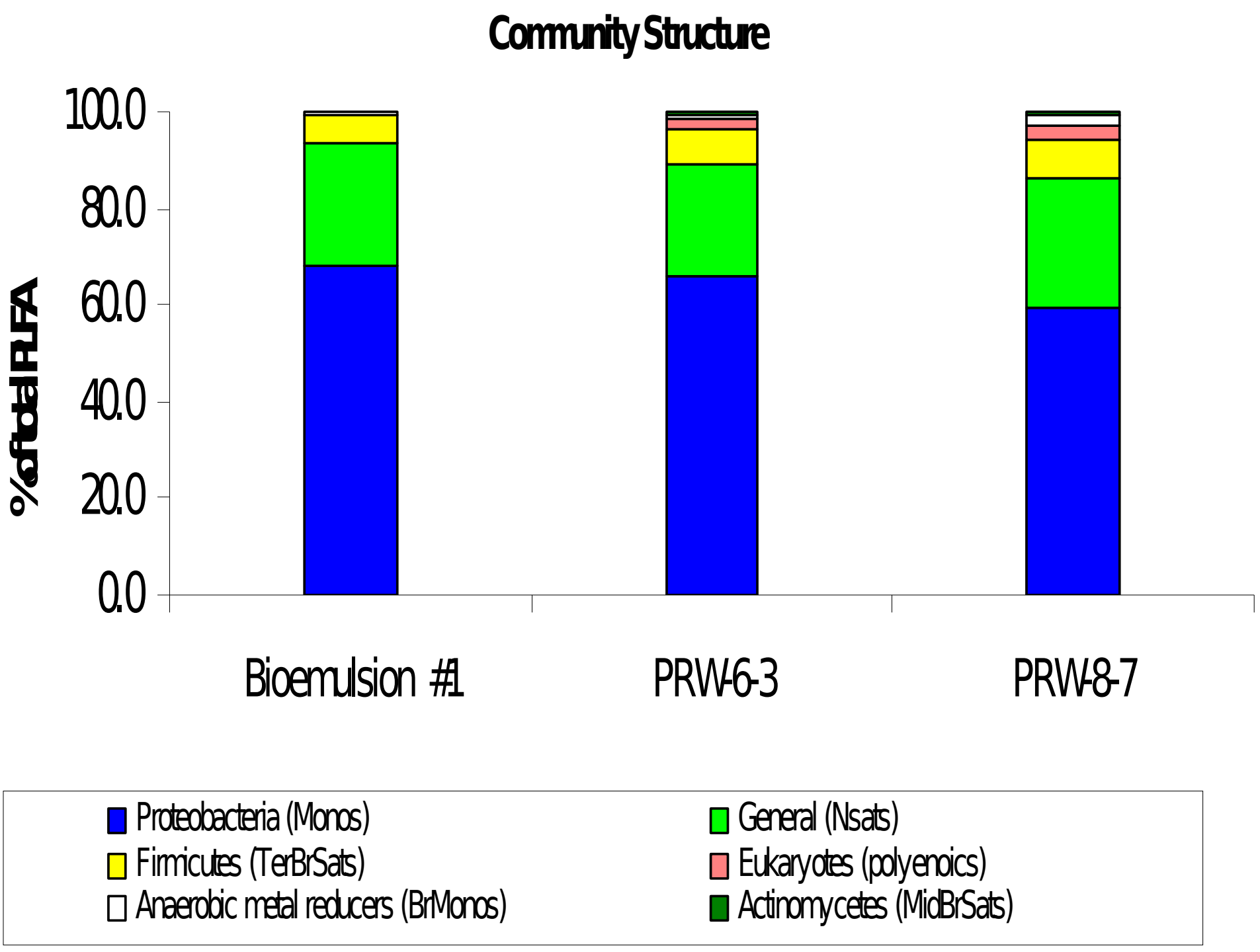
25.9% **Found in all organisms.**

5.3% **Firmicutes (i.e., *Bacillus* & *Clostridium*).**

0.4% **Anaerobes and microaerophiles (*Zoogloea* & *Sulfuricurvum*).**

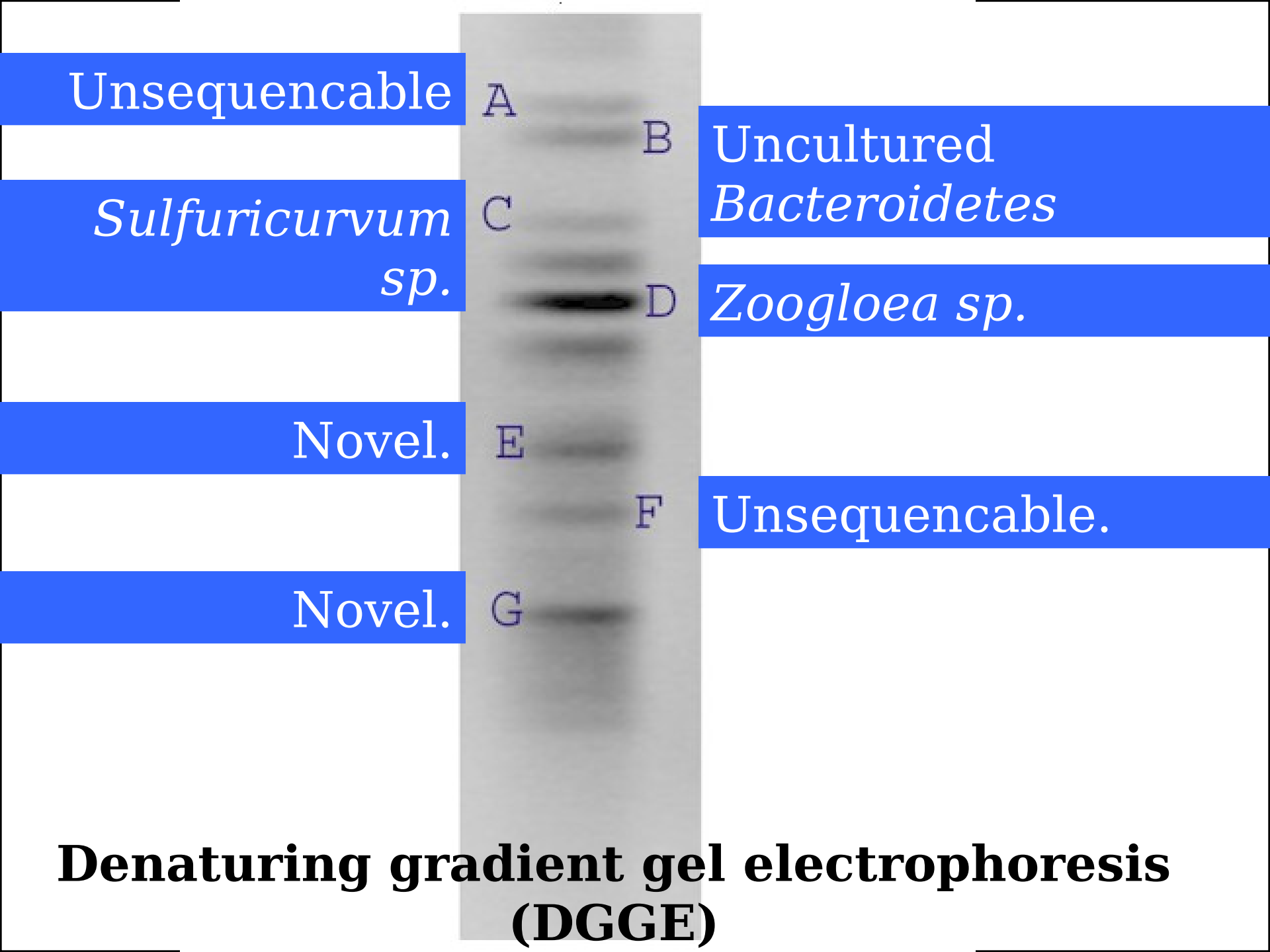
0.3% **Actinomycetes.**

0.3% **Eukaryotes.**

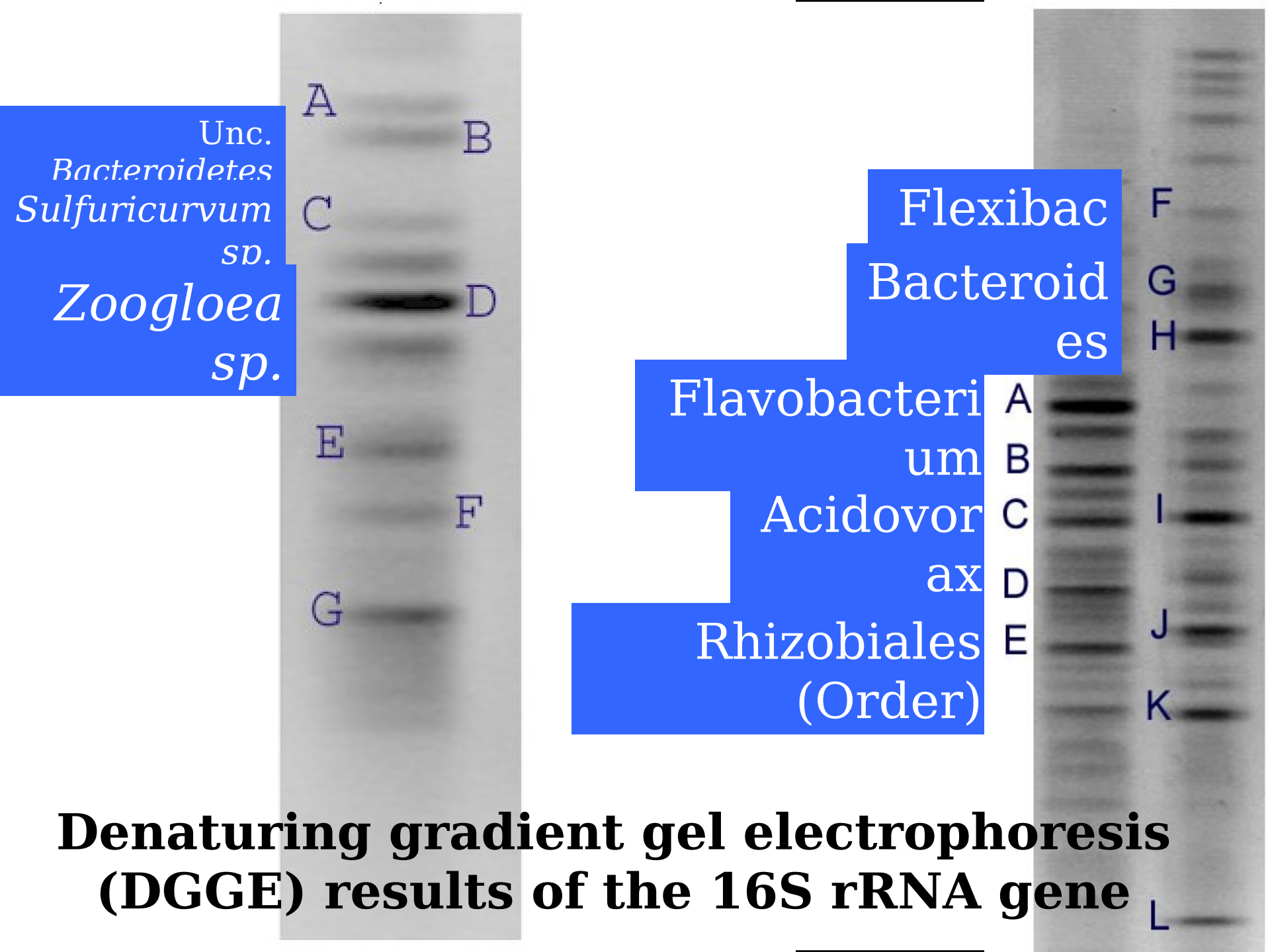

Community Structure
100.0
80.0
60.0
40.0
20.0
0.0
% of total PLFA
Bioemulsion #1
PRW-6-3
PRW-8-7
Proteobacteria (Monos)
General (Nsats)
Firmicutes (TerBrSats)
Eukaryotes (polyenoics)
Anaerobic metal reducers (BrMonos)
Actinomycetes (MidBrSats)

DNA Analysis

- 16S rRNA genes are found in all organisms from bacteria to higher organisms.
- The 16S rRNA gene sequences differ between species.
- 16S rRNA sequences are available on databases to use for identification purposes.



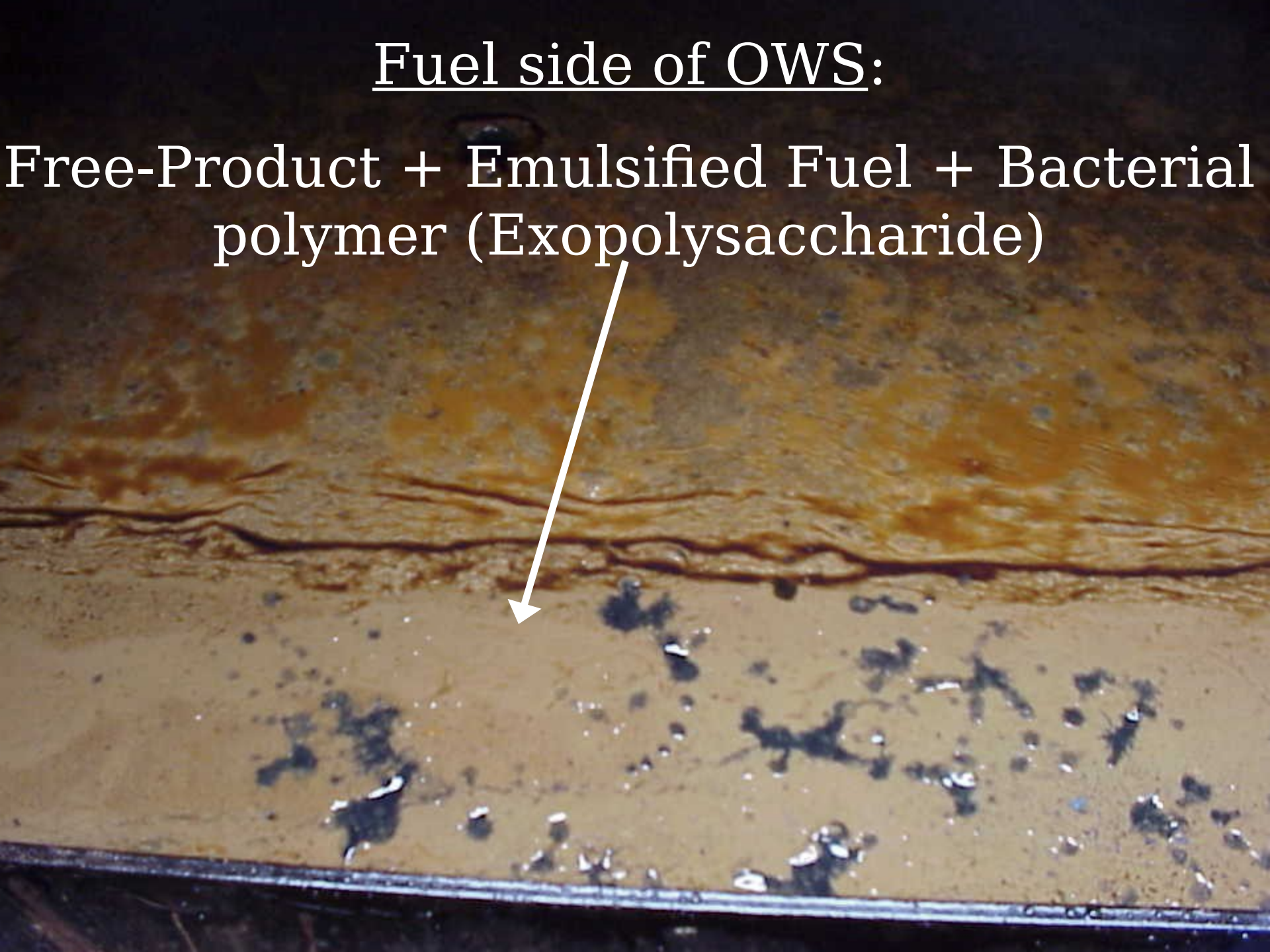
Denaturing gradient gel electrophoresis (DGGE)



Denaturing gradient gel electrophoresis (DGGE) results of the 16S rRNA gene

Exopolysaccharide :

- Bacterial polymer excretion from *Zoogloea,* an aerobic slime forming bacteria.
- Anchors organisms.
- Protects cells from fuel.
- Helps dissolve fuel into an aqueous


Fuel side of OWS:
Free-Product + Emulsified Fuel + Bacterial polymer (Exopolysaccharide)

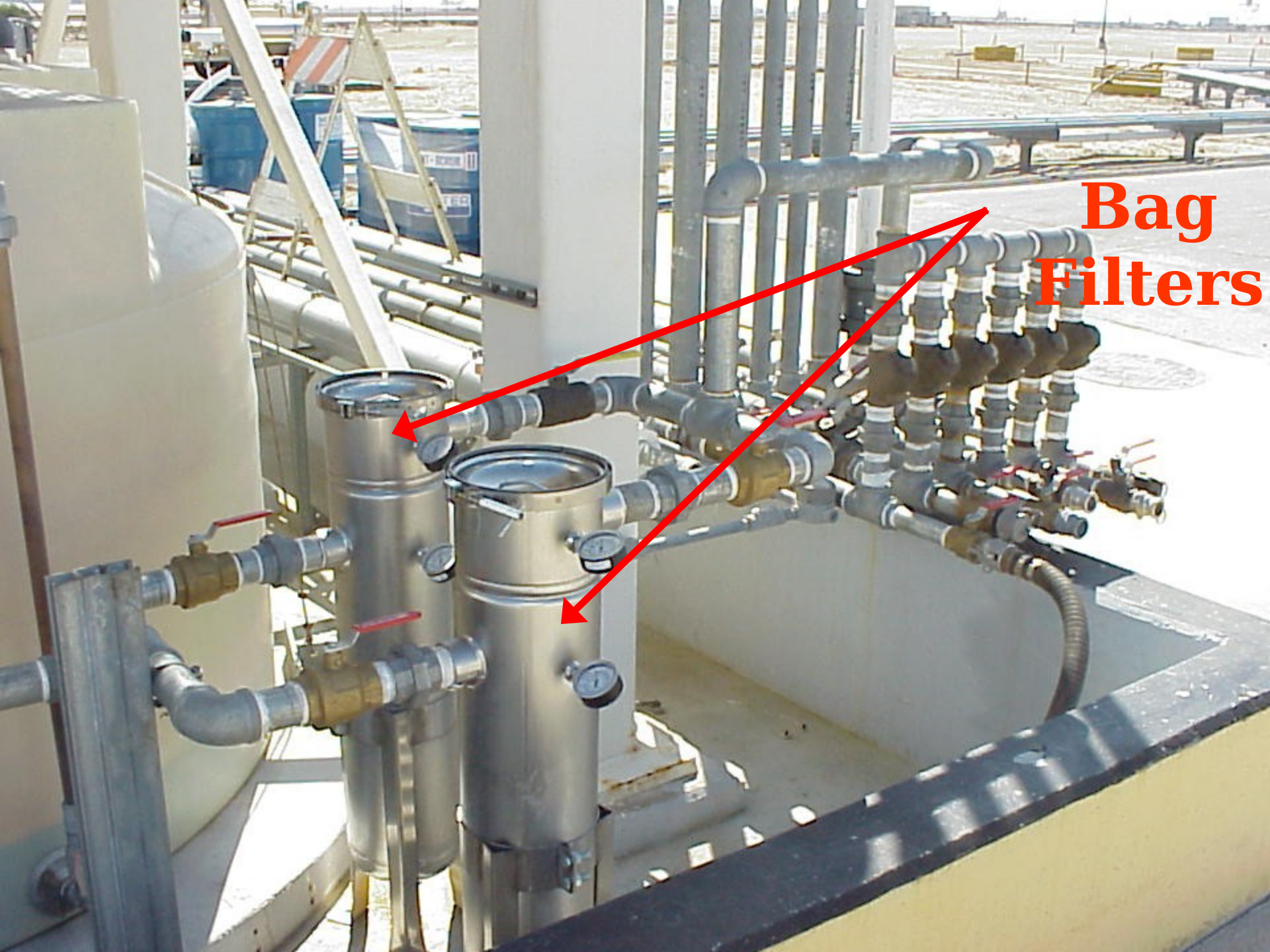
DNA/PLFA Conclusions

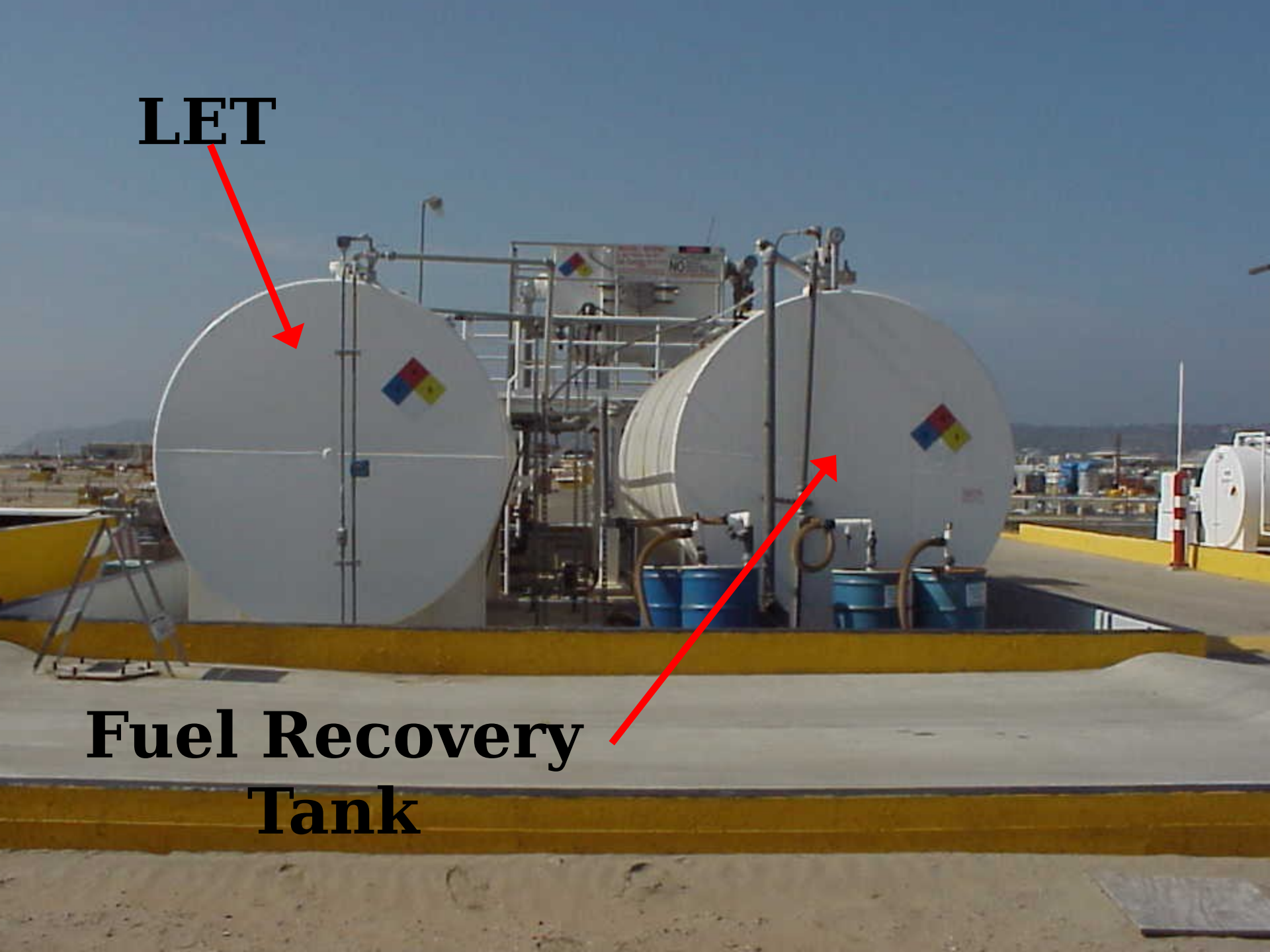
- **Zoogloea dominated community in above-ground treatment system.**
- **No Zoolgoea in detected in subsurface/wells.**
- **Bioemulsion representative of OWS environment, not subsurface environment.**
- **Observed bioemulsion is a microbial excretion: Complex carbohydrates or “Exopolysaccharide ”.**

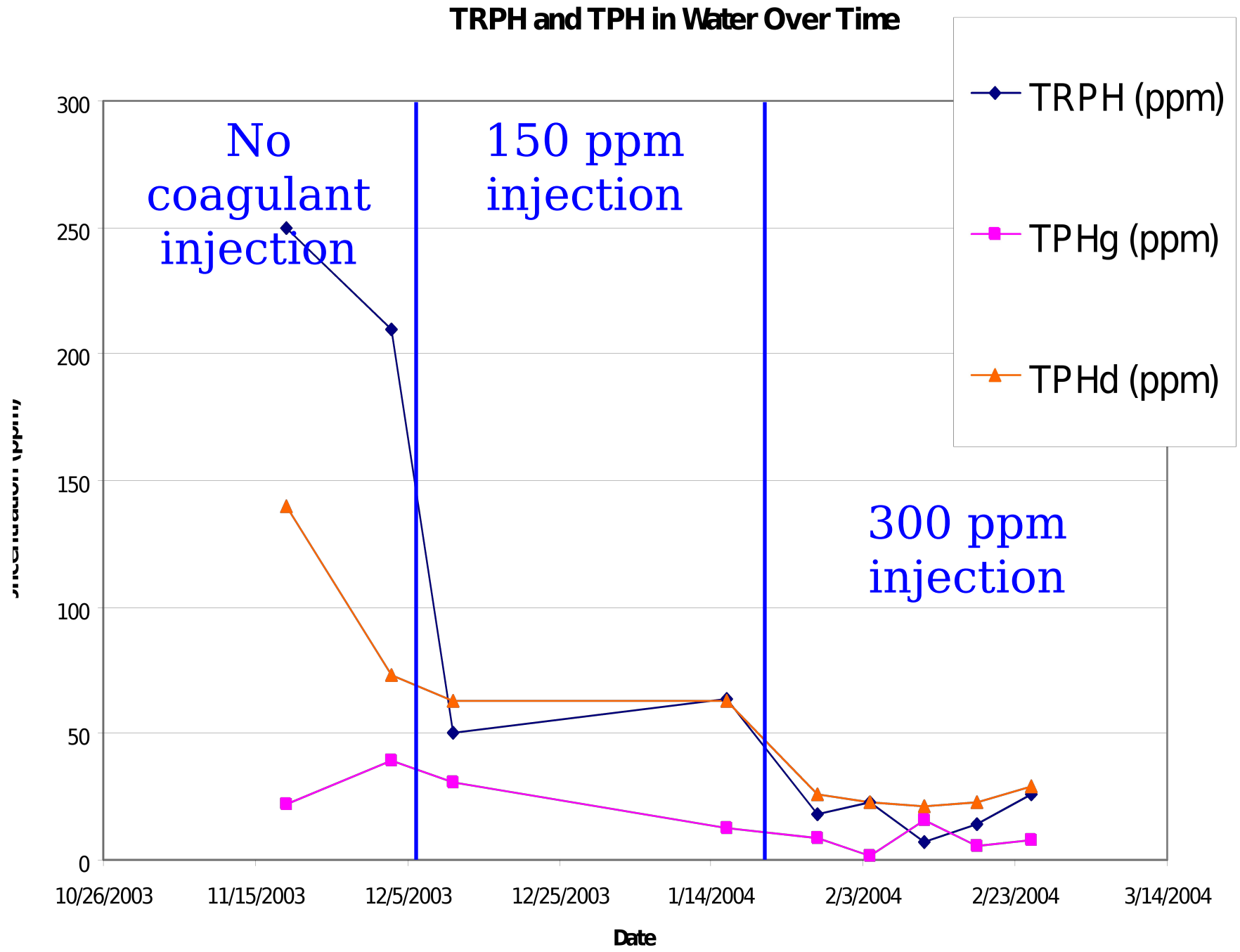
Solutions

Controlling Bioemulsions

1. **In 1999, installed a 12,000-gallon LET and 2 bag filters (in parallel) to increase residence time and collect bioemulsions. Low shear progressive cavity pumps also installed in system.**
2. **In 2003, added “fuel clarifier” to recovered fuel tank to break apart accumulated bioemulsions within recovered fuel.**
3. **In December 2003 to March 2004, tested cationic coagulant.**
4. **Recommend running coagulant inject permanently.**


Bag
Filters


LET
Fuel Recovery
Tank


TRPH and TPH in Water Over Time
TRPH (ppm)
TPHg (ppm)
TPHd (ppm)
No coagulant injection
150 ppm injection
300 ppm injection
300
250
200
150
100
50
0
10/26/2003
11/15/2003
12/5/2003
12/25/2003
1/14/2004
2/3/2004
2/23/2004
3/14/2004
Date

Vendor/ Product	Cost	Purpose	Dose	Annual Use*	Annual Cost*	Other
Emulsion Controls ECO-47 Fuel Clarifier	$9.74 Gallon	Fuel Clarifier	2,000 ppm (2 gal per 1,000 gal Fuel)	66 gallons	$642.84	Fuel Only- for Recovered fuel tank only.
Emulsion Controls ECA-569 Demulsifier	$9.42 Gallon	Demulsifier	500 ppm (1/2 gal per 1,000 gal water)	5,120 gallons	$48,232.64	Needs pH change
Dober Chemical DWT-6150	$7.04 Gallon	Demulsifier	300 ppm (0.30 gal per 1,000 gal water)	3,072 gallons	$21,627.88	No pH change required.

* Assume 2002 water and fuel recovery rates of 10,240,476 gallons and 33,110 gallons, respectively.

Results

- **Able to meet sewer discharge requirements for treated groundwater using LET and bag filters.**
- **Able to eliminate bioemulsion pumping and sludge problems in the fuel recovery tank since January 2003 using fuel clarifier additive.**
- **Total fluids additive show strong reductions in bioemulsion accumulations in LET and OWS and a higher degree of oil/water separation.**

Acknowledgments

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